IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Miyata et al.
Appl. No.	:	10/550,224
Filed	:	May 2, 2006
For	:	COMPOSITION FOR PROMOTING PRODUCTION OF TYPE I COLLAGEN AND/OR ELASTIN
Examiner	:	Nabila G Ebrahim
Group Art Unit	:	1618

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

We, Tetsuhito Sakurai and Youko Handa, are co-inventors of the above-identified application and hereby declare as follows:

1. The following experiments were conducted by us or under our supervision in order to demonstrate the wrinkle-reducing effect of an oral supplement containing silymarin.

2. Test Method

2.1. Preparation of Oral Supplement Containing Silymarin

An oral supplement containing silymarin, and a placebo oral supplement not containing silymarin, both in a soft capsule form, were prepared according to the compositions shown in the table below. Silymarin extract was added by 77 mg per capsule (equivalent to 50 mg of silymarin), and grape seed oil and beeswax were added as excipients, to make soft capsules with a content per capsule of 329 mg. As a placebo, soft capsules having a similar external shape as the silymarin supplement were used.

Ingredient	Oral supplement containing silymarin	Placebo oral supplement

	Content per capsule (mg)	Content per capsule (mg)
Silymarin extract (Silymarin content: Approx. 65%) (Silybin content: Approx. 30%)	77 (50) (23)	-
Grape seed oil	111	160
Beeswax	12	15
Content (Total weight including the capsule)	198 (329)	175 (307)

2.2. Continuous Use of Oral Supplement Containing Silymarin and Placebo Oral Supplement

2.2.1. Test Period and Subjects

Test period: The test was conducted for 16 weeks from March through August of 2004.

Subjects: Females aged 40 years and above (63 subjects)

2.2.2. Design of Test

Subjects were selected randomly from among healthy volunteers and divided into two groups, one to be using the oral supplement containing silymarin and the other to be using the placebo oral supplement, by ensuring an even distribution in terms of age and line condition (number of lines, maximum line depth and overall volume ratio of lines).

- Group using the oral supplement containing silymarin: 31 subjects; average age of 49 years old
- Group using the placebo oral supplement: 32 subjects; average age of 51 years old

2.2.3. Method of Use of Oral Supplement

During the test period, the subjects were asked to take three capsules twice a day after breakfast and dinner, every day.

2.3. Measurement of Number of Lines, Maximum Line Depth and Overall Volume Ratio of Lines

2.3.1. Collection of Line Replicas and Measurement of Number of Lines, Maximum Line Depth and Overall Volume Ratio of Lines

After washing her face and waiting for an adaptation period of 10 minutes in a thermostatic chamber adjusted to a constant temperature of 25°C and constant humidity between 28 and 35%, each subject attached a replica-collecting material

(Skin Cast manufactured by Yamada Cosmetic Laboratories) at the corner of an eye, waited for 15 minutes, and then removed the hardened material (replica) for use in analysis. Replicas were collected before the start of use of each oral supplement (week 0) and after using the oral supplement (week 16), and analyzed using the three-dimensional skin analysis system ASA-03 (manufactured by Asahi Biomed). The method of analysis using this analysis equipment is as follows. To be specific, parallel light was irradiated onto the collected replica at a degree of 30° to capture a grayscale image reflecting the shapes of lines with a CCD camera, and the captured image was loaded onto a computer and processed. The analysis result, or volume of a line, was calculated by "Line Width x Line Depth x 1/2" by approximating a line as an isosceles triangle.

2.3.1.1. Measurement of Number of Lines

A straight line was drawn vertically to the direction of lines and the number of lines crossing the straight line was measured to obtain the number of lines per unit length.

2.3.1.2. Measurement of Maximum Line Depth

The depth of the deepest line in the measured area was used as the maximum depth.

2.3.1.3. Measurement of Overall Volume Ratio of Lines

The volume of lines per unit area was divided by 100 to obtain the overall volume ratio of lines.

3. Measurement Results

3.1. Number of Lines

The results are shown in the table below.

Before use of oral supplement

	Average number of lines Lines/mm	Standard error Lines/mm	p-value
Group using the oral supplement containing silymarin	0.197	0.024	0.908
Group using the placebo oral supplement	0.193	0.020	

After use of oral supplement (after 16 weeks)

	Average number of lines Lines/mm	Standard error Lines/mm	p-value
Group using the oral supplement containing silymarin	0.163	0.018	0.108
Group using the placebo oral supplement	0.212	0.024	

The t-test of the average numbers of lines on the subjects in the group using the oral supplement containing silymarin and group using the placebo oral supplement, before use of oral supplement, found the p-value to be 0.908, with no difference found between the two groups.

On the other hand, the average number of lines after 16 weeks of use of oral supplement decreased on the subjects in the group that used the oral supplement containing silymarin. The t-test of the average numbers of lines on the subjects in the group using the oral supplement containing silymarin and group using the placebo oral supplement resulted in a p-value of 0.108, and the number of lines decreased substantially more on the subjects in the group that used the oral supplement containing silymarin, compared to the subjects in the group that used the placebo oral supplement.

3.2. Measurement of Maximum Line Depth

The results are shown in the table below.

Before use of oral supplement

	Average maximum line depth μm	Standard error μm	p-value
Group using the oral supplement containing silymarin	202.6	7.7	0.656
Group using the placebo oral supplement	208.5	10.8	

After use of oral supplement (after 16 weeks)

	Average maximum line depth μm	Standard error μm	p-value
Group using the oral supplement containing silymarin	190.3	6.8	0.113

Group using the placebo oral supplement	209.2	9.7	

The t-test of the average maximum line depths on the subjects in the group using the oral supplement containing silymarin and group using the placebo oral supplement, before use of oral supplement, found the p-value to be 0.656, with no difference found between the two groups.

On the other hand, the average maximum line depth after 16 weeks of use of oral supplement decreased on the subjects in the group that used the oral supplement containing silymarin. The t-test of the average maximum line depths on the subjects in the group using the oral supplement containing silymarin and group using the placebo oral supplement resulted in a p-value of 0.113, and the maximum line depth decreased substantially more on the subjects in the group that used the oral supplement containing silymarin, compared to the subjects in the group that used the placebo oral supplement.

3.3. Measurement of Overall Volume Ratio of Lines

The results are shown in the table below.

Before use of oral supplement

	Overall volume ratio of lines $\mu m^3/mm^2/100$	Standard error $\mu m^3/mm^2/100$	p-value
Group using the oral supplement containing silymarin	97.2	63.7	0.955
Group using the placebo oral supplement	98.0	47.3	

After use of oral supplement (after 16 weeks)

	Overall volume ratio of lines $\mu m^3/mm^2/100$	Standard error $\mu m^3/mm^2/100$	p-value
Group using the oral supplement containing silymarin	79.6	56.0	0.197
Group using the placebo oral supplement	97.0	43.5	

The t-test of the overall volume ratios of lines on the subjects in the group using the oral supplement containing silymarin and group using the placebo oral supplement, before use of oral supplement, found the p-value to be 0.955, with no difference found between the two groups.

On the other hand, the average overall volume ratio of lines after 16 weeks of use of oral supplement decreased on the subjects in the group that used the oral supplement containing silymarin. The t-test of the average overall volume ratios of lines on the subjects in the group using the oral supplement containing silymarin and group using the placebo oral supplement resulted in a p-value of 0.197, and the overall volume ratio of lines decreased substantially more on the subjects in the group that used the oral supplement containing silymarin, compared to the subjects in the group that used the placebo oral supplement.

4. I hereby declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Dated: 7, 18, 2008 By: Tetsuhito Sakurai
Tetsuhito Sakurai

Dated: 7, 22, 2008 By: Youko Handa
Youko Handa